AUSTRALIAN RUST STUDIES. VI.

COMPARATIVE STUDIES OF BIOTYPES OF RACE 34 OF PUCCINIA GRAMINIS TRITICI.

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(Plate xi.)

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Introduction.

Physiological specialization is now known to occur frequently in parasitic fungi. It reaches a high development in the plant rusts. The determination of physiological races within the cereal rusts has received a great deal of attention in many countries, based largely on methods developed at the Minnesota Agricultural Experiment Station by Stakman and Levine (1938). Using this technique, more than two hundred races of wheat stem rust have been determined.

In Australia, in work which has extended over the past twenty years, nine races have been found (Waterhouse, 1939). Since 1929 approximately 98% of the isolates examined have proved to be race 34, a rust which is also well known in other countries. In this determination work, and in studies of race 34, it was early found (Waterhouse, 1929) that changes in the environmental conditions caused marked variations in the reactions shown by certain of the differential hosts that are normally used. In particular, the varieties Arnautka, Mindum and Spelmars at temperatures of 70–75°F. exhibited reactions of type 3 and type 4, but at about 55°F. the reactions were flecks. That is to say, what was race 34 at the higher temperatures became race 56 at the lower. Other varieties exhibit changes in reaction of this same nature. The culture used in these studies is the one which was first found in November, 1925. It has since been maintained in the uredospore stage, and is the one referred to herein as the standard Australian biotype.

INVESTIGATIONS IN U.S.A.

Recently it became possible to make comparisons between the Australian race 34 and an American culture of the same designation. One of us (I.A.W.) had opportunities of carrying out the investigations whilst working under Dr. E. C. Stakman at the Minnesota Agricultural Experiment Station.

(*a*). *Tests with Fixed Crossbred Wheats.*

During the spring and summer of 1939, a number of rust resistant selections were tested in the rust nursery at St. Paul, Minnesota. These included derivatives of Kenya wheats and others which owe their resistance to the variety of *Triticum durum* named "Gaza". All had been selected and tested for their rust resistance in the plant-house and under field conditions in New South Wales where race 34 was present. Of thirty-one lines, five segregated for resistance and susceptibility. Race 34 was isolated from susceptible pustules in segregating lines.

If independent genetic factors were responsible for resistance to the U.S.A. and the Australian biotypes of race 34, it would be impossible to select consciously, lines that are resistant to both biotypes, although such lines could be isolated by chance. There was no evidence that two different genes control resistance to the two biotypes, especially since so few discrepancies occurred in the tests that were made.

(*b*). *Tests with F_3 Lines.*

Fifty-five F_3 lines of the cross Dundee 985* × Kenya 745 and thirty of the cross Federation 107 × Kenya 745 were selected at random. The progeny of each single F_2 plant was divided into three parts. One part was sown and inoculated at St. Paul in the seedling stage with a U.S.A. culture of race 34. A second part was sown in the

* Varieties carry the Sydney University Accession Number.

field at St. Paul and exposed to an epiphytotic caused by several races of stem rust. The third part was sent to the University of Sydney, where the seedlings were inoculated with the standard culture of the Australian race 34.

In Table 1 are given the results of the seedling inoculations in the two countries.

TABLE 1.

Reactions of F_3 lines and the parents of two crosses, Dundee 985 × Kenya 745 and Federation 107 × Kenya 745, when inoculated with the two biotypes of race 34 of Puccinia graminis Tritici.

	F_3 Reactions to U.S.A. Biotype.								Origin of Culture.	Parents.		
	Dundee 985 × Kenya 745.				Fed. 107 × Kenya 745.							
	Res.	Seg.	Susc.	Total.	Res.	Seg.	Susc.	Total.		Dundee 985.	Kenya 745.	Fed. 107.
Reactions to the Australian Biotype—												
Res. ..	10			10	9			9	United	3+	; and 2	4
Seg. ..		31		31		15		15	States.			
Susc. ..			14	14			6	6	Australia.	3	;	3+
Total ..	10	31	14	55	9	15	6	30				

It is clear that the same gene was found to govern seedling resistance to both cultures, irrespective of whether Kenya 745 was crossed with Federation 107 or Dundee 985. Parental varieties showed slight differences, all three indicating that the U.S.A. culture of this race is more virulent than the standard Australian culture. The F_3 results show that one and the same gene governs resistance to both cultures of race 34. The seedling reactions at St. Paul showed complete correlation with the mature plant behaviour in the field there.

(*c*). *Tests with Inbred Ryes.*

In 1929 a number of selfed single plants of rye were selected in Australia on the basis of their field reaction to Australian race 34. Progenies have been tested each year, grown on and selfed. The selections came from the varieties "March" and "Petkus Rug". Their usefulness as differentials of *P. graminis Secalis* has already been demonstrated (Waterhouse, 1938). The reactions of these lines to the Australian race 34 are stable, some being resistant, others susceptible.

Table 2 gives the results of testing thirty-one of these lines, whose reactions to the Australian culture were known, with a U.S.A. biotype of race 34.

TABLE 2.

Reactions of thirty-one inbred lines of rye to two biotypes of race 34 of P. graminis Tritici.

Inbred.	Origin of Rust Culture.		Inbred.	Origin of Rust Culture.	
	United States.	Australia.		United States.	Australia.
R_2	;	3	R_{18}	3	3
R_3	x = and ;	3	R_{19}	3	3
R_4	; and 1+	3	R_{20}	x	3
R_5	1+	3	R_{21}	3	3
R_6	; and 1+	3	R_{22}	3	3
R_7	; and 1+	3	R_{23}	3	3
R_8	;	3	R_{24}	;	2=
R_9	x=, ; and 1	3	R_{25}	;	2=
R_{10}	;	3	R_{26}	;	x
R_{11}	x	3	R_{27}	;	x
R_{12}	3	3	R_{28}	;	x
R_{13}	3	3	R_{41}	x	;
R_{14}	3	3	R_{42}	3 and x	;
R_{15}	3	3	R_{43}	;	;
R_{16}	3	3	R_{44}	x+, ; and 1	;
R_{17}	3	3			

It is apparent that selection over a period of years, either for resistance or susceptibility to one biotype of race 34, does not necessarily insure that the selection so made will give the same reaction with another collection of the same race. Whereas the same genetic factor was concerned in the reactions shown by the Kenya 745 cross-breds, in the inbred ryes different factors govern the resistance to the two biotypes of race 34.

(*d*). *Tests on Standard Differentials.*

Arising out of the finding (Waterhouse, 1929) that the Australian race 34 gives the reactions of race 56 at low temperatures at Sydney, a study was made to find out how closely a U.S.A. culture of race 34 resembled race 56 at low temperatures at Minnesota.*

Two random isolates, one representing race 34 and the other race 56, were made from the United States Department of Agriculture field survey material. Each was increased on Little Club and the experiment made during the autumn of 1939, when two suitable average temperatures were available. The lower temperature varied between 53·6°F. and 68°F., with an average of 58°F., and the higher varied between 64°F. and 77·5°F., with an average of 70·5°F. At each of these two temperatures three light intensities were used. The highest intensity of 1,600 foot candles was the average when plants were exposed to sunlight on the greenhouse bench. A medium intensity of 500 foot candles and a low intensity of 300 foot candles were obtained by placing the seedlings beneath cages covered by two and four thicknesses respectively of 80-mesh cheesecloth.

Plants of Kota, Arnautka, Mindum, Spelmars, Kubanka, Acme, Einkorn, Hope, and Thatcher were inoculated, when two inches high, by the brush method. Notes were taken fifteen days after inoculation. The results are set out in Table 3.

It is clear that in general the lower temperature tends to depress the severity of the rust attack, and browning, previously reported by Hart and Allison (1940), occurs on some varieties at the higher temperatures. Despite a reduction in severity of reaction, however, Arnautka, Mindum and Spelmars do not approach the fleck reactions with U.S.A. race 34 as reported for the Australian race 34 at similar temperatures. There is therefore no possibility of confusing the U.S.A. races 34 and 56 even at low temperatures.

INVESTIGATIONS IN AUSTRALIA.

Thanks to the courtesy of the Matson Navigation Co., whose officials refrigerated a collection of uredospores of U.S.A. race 34 whilst *en route* to Australia, a culture from Minnesota became available for studies carried out in Sydney. Side by side comparisons of the reactions shown by the American and Australian rusts thus became possible.

At the outset the two cultures were checked for their reactions on the usual set of differentials at a temperature of about 75°F. on the plant-house bench at the University of Sydney. During May and June, 1940, preliminary comparisons were made of the two cultures, and in July and August the studies were carried out in greater detail.

For purposes of the experiment, two different temperatures and two different exposures to light were used. Because of limitations of space, only some of the twelve usual differentials were employed, each pot containing fifteen to twenty seedlings. Some fluctuations occurred in the temperatures during the tests, but the average readings were 75°F. in the one house and 55°F. in the other. Artificial light was provided for the long day series.

The results are summarized in Table 4.

It will be seen that there are striking differences between the two biotypes, as measured by the reactions shown by the differentials. Typical reactions are illustrated in Plate xi. The U.S.A. race 34 is much more virulent. On Kota, the Australian race at 75°F. gives somewhat stronger reactions, but on the durums the differences are very marked in the other direction. The resistant reactions on Arnautka, Mindum and Spelmars at the low temperature are in agreement with the observations previously reported, and regularly found year after year during the winter months where the

* Grateful acknowledgement is made to Mr. W. Q. Loegering for help in conducting this experiment.

TABLE 3.

Reactions of varieties of wheat to two races of P. graminis Tritici *at two temperatures and three light intensities.*

Variety.	High Temperature.						Low Temperature.					
	Light Intensity and Race No.						Light Intensity and Race No.					
	High.		Medium.		Low.		High.		Medium.		Low.	
	34	56	34	56	34	56	34	56	34	56	34	56
Kota ..	3− b	3, 4−	3= b	3−, 3+	3= b	3	3−	3	3=, 3	3=, 3+	3=	3=, 3
Arnautka ..	3, 4	0, ;, 1,	3, 4−	0 ; 1	3	0 ; 1=	3−, 3	0 ; 1	3, 3+	0 ; 1	3=, 3	0 ;
Mindum ..	4	0 ; 1	3, 4	0 ; 1	3, 3+	0 ; 1=	3	0 ; 1	3−, 3++	0 ; 1	3=, 3	0 ; 1
Spelmars ..	4+	0 ; 1	3, 4	0 ; 1	3, b=	0 ; 1	3=, 3+	0 ; 1	3, 3++	0 ; 1	3−, 3	0 ;
Kubanka ..	3, 4 b=	3, 4	3	3, 4	3	3−, 3	3	3	3−, 3++	3−, 3++	3=, 3	3=, 3
Acme ..	3, 4 b	3+, 4	3 b	3 b	3= b	3	3+	3=, 3	3, 3+	3= 3+	3=, 3	3=, 3
Einkorn ..	0 ; 1=	0 ; 1	0 ; 1−	0 ; 1=	0 ;	0 ;	0 ; 1	0 ; 1++	0 ; 1	0 : 1++	0 ;	0 ; 1−
Hope.. ..	3, 4	1+, 2−	3, 4	1, 2=	3, 3+	0 ; 2−	3	0 ; 1++	3−, 3++	0 ; 1++	3, 3+	2=
Thatcher ..	x+ b	x+	x+ b−	x++	3 b	3, 3+	x=	x−	x−	x−	x=	x−

b represents browning.

b= represents faint browning.

TABLE 4.

Reactions of two biotypes of race 34 of P. graminis Tritici *on wheat differentials, when tested side by side at two temperatures and two light exposures.*

Variety.	Temperature 75° F.				Temperature 55° F.			
	18 Hours Light.		6 Hours Light.		18 Hours Light.		6 Hours Light.	
	U.S. 34.	Aust. 34.	U.S. 34.	Aust. 34.	U.S. 34.	Aust. 34.	U.S. 34.	Aust. 34.
Kota	2+	3+	2	3	2+	2	2−	2=
Arnautka	4	3+	3	3− and x−	3++	3, 1 and ;	3−	3=, 1 and ;
Mindum	4	3+ and x+	3	3− and x−	3++	1 and ;	3−	1 and ;
Spelmars ..	4	3+ and x+	3	3− and x−	3++	1 and ;	3−	1 and ;
Kubanka	3 and 3c	3c and x	3−c	x−	3 and x	x−	x−	x=
Acme	3 and 3c	3c and x	3−c	x−	3 and x	x	x−	x=

standard Australian race is used, as well as in cases where other isolates of Australian race 34 have come up for observation.

Comparisons of the two biotypes showed that there were differences in the colour of their uredosori. On the Ridgway Colour Standards the U.S.A. biotype is "Burnt Sienna of Plate II", whilst the Australian biotype is "Sanford's Brown of Plate II". For long we have had marked colour differences between *different* physiological races, some of which have already been reported (Waterhouse, 1929), and from the standard Australian culture of race 34, a very light colour variant (Ridgway Standard "Light Cadmium of Plate IV") has arisen on three different occasions during the past fifteen years. Its reactions on the differential hosts have been similar to those of the parent culture.

Not only in colour have differences been found in different isolates of Australian race 34. Thus on the variety "Barwang", which gives type 3+ reactions with the standard culture, an isolate which came from South Australia gave flecks, and yet when tested on the twelve differentials, it gave typical race 34 reactions.

Passing reference may also be made to results which will be reported in fuller form later. Cultures derived from barberries inoculated with race 34 have received considerable study. In them, it has been found that isolates that gave similar reactions of race 34 at 75°F. differ in their response to lower temperatures, as measured by their resistant reactions on differentials like Arnautka, Mindum and Spelmars.

Again, it has been found that comparisons at 70-75°F. of the U.S.A. and the Australian biotypes on such varieties as Marouani, D5, Nodak, Pinet, Trigo Africano, Egypt 75 and Greece 18, reveal marked differences in the reactions shown. The implications of this in respect of a programme of breeding for rust resistance are very important.

This position is similar to that which occurs in *Puccinia triticina*, where it has been shown (Waterhouse, 1929) that Australian isolates tested on the eight normal differentials give similar reactions. But if further tested on a variety like "Thew", one isolate may give a completely resistant, and another a completely susceptible reaction. That is to say, two different rusts are involved. Size differences between their uredospores have been demonstrated, and also differences in their prevalence at different times during the season. The fact that there are really two rusts obviously has an important bearing on the problem of breeding for resistance.

It is certain that if more differential varieties were added to the twelve now generally used to determine races of *P. graminis Tritici*, many more races could be isolated. Aamodt (1934) has given the formula for calculating the number of races

possible with any one group of varieties, disregarding the mesothetic reaction. The advisability of thus increasing the number of stem rust differentials, however, is questionable for general practice. For special purposes it may be desirable.

Conclusion.

These results show that the normal method of sorting out physiological races by means of the reaction shown on certain differential hosts that have been empirically selected, does not necessarily sort out entities that are identical. Physiological races which bear the same designation may be different. Thus the U.S.A. race 34 is not the same as the Australian race 34. Clearly physiological races do not represent the final stages in the analysis of a rust culture. A term is necessary to define these entities. "Isolates" would cover the requirement, but has a rather wider application than is wanted. It is believed that the term "biotype" as suggested by Christensen and Rodenhiser (1940) for the smut fungi adequately describes these entities. A biotype, then, is a single individual or group of individuals having the same genetic constitution. In the case of *P. graminis*, two isolates may or may not be the same biotype. Where hybridization on *Berberis* sp. is common, it may be expected that a physiological race will comprise a number of biotypes; but under Australian conditions where barberries are not frequently infected, race 34 probably comprises relatively few biotypes, which have arisen mainly in the asexual stage.

Summary.

Comparisons, on a cultural basis, have been made between a culture of *Puccinia graminis Tritici* isolated in U.S.A. and a standard Australian culture of the same race, first at St. Paul, Minnesota, and secondly at the University of Sydney.

1. At St. Paul:
   - (*a*). Fixed Australian crossbreds gave no reliable proof that the biotypes are different.
   - (*b*). F_3 lines from two crosses which had been tested in Australia with race 34 showed similar reactions when tested with a U.S.A. biotype of this race.
   - (*c*). Inbred ryes of known reactions to the Australian biotype gave quite different reactions when tested with the U.S.A. biotype.
   - (*d*). Differential varieties recorded as showing a changed reaction to race 34 in Australia, owing to a lowered temperature, did not thus change when tested with the U.S.A. biotype.
2. At the University of Sydney, side by side comparisons of the two biotypes were made under varying conditions of temperature and light:
   - (*a*). Differential varieties, previously reported to give a change in reaction with a lowered temperature, did thus change when tested with the Australian biotype, but did not with the U.S.A. biotype.
   - (*b*). Colour differences are discernible between the two biotypes.
   - (*c*). On certain varieties not included in the set of differentials, the two biotypes show different reactions.

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EXPLANATION OF PLATE XI.

Leaves of Mindum showing (A) the susceptible reactions to the U.S.A. biotype and (B) the resistant reactions to the Australian biotype of race 34 of *P. graminis Tritici*. The plants were exposed to 18 hours' light at a temperature of 55°F. In each, two leaves show the upper and two the lower surfaces of the leaves. Nat. size × 2.